

Applicant : Klaus Cichutek *et al.*
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REMARKS

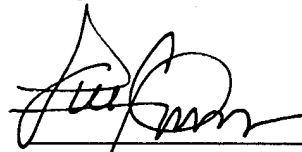
The present amendment is made to replace the Sequence Listing filed with the application with the Sequence Listing filed herewith and to insert sequence identifiers into the text of the specification, where appropriate. No new matter has been added.

Attached is a marked-up version of the changes being made by the current amendment.

No fees are believed due in connection with this amendment. If there are any fees, or any credits, please apply them to Deposit Account No. 06-1050.

Respectfully submitted,

Date: January 17, 2002



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Version with markings to show changes made

In the specification:

The paragraph beginning at page 5, line 12, has been amended as follows.

[Fig. 4 shows] Figs. 4A and 4B show the nucleic acid sequence of pTC53 (SEQ ID NO:1) and amino acids encoded thereby (SEQ ID NOs:2-21, 23-31, and 22, respectfully).

The paragraph beginning at page 9, line 32, has been amended as follows.

2. Cloning of specific scFv-cDNA fragments into Env expression constructs

The scFv-cDNAs of 150 cell-specific scFv were excised in a usual manner from the phagemid DNA and each of the DNAs was ligated into the expression construct pTC53. pTC53 was obtained by modification of the universal eukaryotic vector pRD114 (Chu et al., J. Virol. 71 (1997) 720-725; Sheay et al., BioTechniques 15 (1993) 856-861; Chu et al., BioTechniques 18 (1995) 890-895). In this vector, the SNV-wt-env gene was deleted except for the leader sequence and the transmembrane-protein encoding cDNA. An additionally inserted spacer enables the insertion of a foreign DNA (here scFv-cDNA) following the ENV-leader sequence via the restriction recognition site NaeI. The sequence of pTC53 is shown in figure 4. For the insertion of the scFv-cDNA the Env-expression construct pTC53 was modified so that Sfi I and Not I specific restriction endonuclease recognition sites and inserted between the SNV-leader sequence and SNV-transmembrane sequence (T M) in a usual manner. For this purpose a recombinant PCR is carried out in a usual manner starting from the DNA of the planned PKA1558 (Scov. H. & Andersen, K.B., 1993) and the DNA coding of the anti-transferrin receptor scFv so that via Nru I (5' and 3') an insertion of the amplified fragment into the Nae I restricted pTC53 is possible. The thus inserted fragment contains the multiple Sfi I/Not I cloning site since the primers used further include a neighboring Sfi I or Not I recognition site, respectively, in addition to the terminal Nru I recognition site. For recombinant PCR the following primers were used (SEQ ID NOs:32 and 33):

The paragraph beginning at page 12, line 22, has been amended as follows.

Preparation of zeocin resistance gene by means of PCR starting from DNA of the plasmid pSCV Zeo (Invitrogen [Comp.] Company): To select packaging cells after a stable transfection with the pTC53-zeo-scFv plasmid for a stable expression of the resistance gene, a zeocin cassette was integrated. For this purpose, a zeocin cassette was amplified by means of recombinant PCR from the vector pZeoSV2 (+/-) of Invitrogen Company (NV Leek, The Netherlands) and provided with the restriction sites NdeI 5' and 3' so that the cassette subsequently could be inserted into the NdeI restricted portion of the pUC19 backbone of pTC53. The PCR-batch (100 µl) contained: 1 x PCR buffer (Taq: 10 mM Tris/HCl, pH 8.8, 50 mM KCl, 1.5 mM MgCl₂, 0.01% gelatin, 10 µM (+)- and 10 µM (-)-primer, 200 µM of each deoxynucleotide, 2.5 units of Taq polymerase and 100 ng of plasmid DNA. [Following] The following oligonucleotides have been used (SEQ ID NOS:34 and 35):